Cerebral Ischemia in Aneurysmal Subarachnoid Hemorrhage
A Correlative Microdialysis-PET Study

Asita S. Sarrafzadeh, MD; Daniel Haux, MD; Lutz Lüdemann, PhD; Holger Amthauer, MD; Michail Plotkin, MD; Ingeborg Küchler, PhD; Andreas W. Unterberg, MD, PhD

Background and Purpose—Cerebral microdialysis (MD) is discussed as a technique for detection of cerebral ischemia in subarachnoid hemorrhage; however, clinical data on cerebral blood flow (CBF) are limited in these patients. The main objective of this study was to investigate whether pathological MD parameters reflect a reduced regional CBF (rCBF) determined by $^{15}$O-H$_2$O PET.

Methods—Thirteen subarachnoid hemorrhage patients (age, 48.7±15.0 years; World Federation of Neurological Surgeons grade 1 to 5) were studied. Extracellular glucose, lactate, lactate/pyruvate (L/P) ratio, glutamate, and glycerol levels were analyzed hourly. rCBF was determined in the volume of interest of the MD catheter and all vascular territories. MD values were correlated to rCBF on the day of PET. Then, MD concentrations of asymptomatic versus ischemic phases (3-day medians) were analyzed.

Results—In symptomatic patients (n=10), rCBF was significantly lower compared with controls (n=3, P=0.048). Glutamate correlated best with rCBF ($r=-0.66; P=0.014$), followed by glycerol ($r=-0.62; P=0.021$). The L/P ratio was most sensitive (0.82) and specific (1.0) in indicating symptoms of ischemia, but only during longer periods of ischemia.

Conclusions—rCBF correlates best with glutamate, followed by glycerol, whereas the L/P ratio is sensitive only after longer periods of ischemia. Clinically relevant regional metabolic derangements occur already above an rCBF of 20 mL·100 g$^{-1}$·min$^{-1}$. Future research should focus on identifying alternative causes of metabolic derangement in subarachnoid hemorrhage patients and optimal treatment management in these patients. (Stroke. 2004;35:638-643.)

Key Words: cerebral blood flow ▪ cerebral metabolism ▪ ischemia ▪ microdialysis ▪ subarachnoid hemorrhage

Subarachnoid hemorrhage (SAH) is still a significant cause of morbidity and mortality in 10 to 16 per 100 000 persons per year. Cerebral ischemia secondary to vasospasm occurs in 20% to 30% of these patients and has been correlated with a 1.5- to 3-fold increase in mortality in the first 2 weeks after SAH. Because cerebral ischemia can be difficult to predict, reliable markers that precede the onset of clinical symptoms are needed to identify patients at risk for this feared complication.

Functional imaging of the brain with PET provides an excellent assessment of cerebral blood flow (CBF). PET studies have shown that CBF and cerebral blood volume are decreased during cerebral vasospasm, which suggests that the appropriate vasodilating capacity of distal vessels in response to local cerebral perfusion pressure is impaired after SAH. However, PET is not feasible for routine clinical use.

Cerebral microdialysis (MD) is a method to sample and analyze online extracellular concentrations of cerebral metabolites at the patient’s bedside. MD concentrations were shown to correlate with the neurological condition in patients with head injury, SAH, stroke, and epilepsy. Extracellular glutamate, lactate, and the lactate/pyruvate (L/P) ratio are discussed as sensitive markers of ischemia in SAH patients. However, whether the extracellular concentrations of MD parameters indicate real changes in CBF or reflect local derangement of cerebral metabolism is unclear. The main objective of this study was to investigate whether pathological MD parameters reflect a reduced regional CBF (rCBF) determined by $^{15}$O-H$_2$O PET. Second, we wanted to identify the sensitivity and specificity of the MD parameters to indicate ischemia comparing asymptomatic and symptomatic intervals in ischemic SAH patients.

Materials and Methods

Ethics
This study was approved by the local research ethics committee at Charité Virchow Medical Clinic and by the German Radioactive Protection Authorities. Written informed consent was obtained from
TABLE 1. Demographic and Clinical Data

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, y</th>
<th>Sex</th>
<th>Admission WFNS</th>
<th>Aneurysm Location</th>
<th>Fisher Score</th>
<th>Symptomatic</th>
<th>PET</th>
<th>Clinical Symptoms at PET</th>
<th>Day of PET After SAH</th>
<th>Location of MD Probe</th>
<th>Vasospasm in Control Angiography/ TCD</th>
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<td>1</td>
<td>22</td>
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<td>MCA R</td>
<td>2</td>
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<td>1</td>
<td>Headache</td>
<td>12</td>
<td>R temp</td>
<td>No/no</td>
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<td>40</td>
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<td>PcomA</td>
<td>2</td>
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<td>1</td>
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<td>No/no</td>
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<tr>
<td>3</td>
<td>72</td>
<td>F</td>
<td>2</td>
<td>ICA L</td>
<td>2</td>
<td>No</td>
<td>1</td>
<td>Mild headache</td>
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<td>L temp</td>
<td>No/no</td>
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<td>4</td>
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<td>M</td>
<td>2</td>
<td>AcomA</td>
<td>3</td>
<td>Yes</td>
<td>1</td>
<td>Aphasia</td>
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<td>R front</td>
<td>Yes, MCA + ACA bilateral/NA</td>
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<td>5</td>
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<td>3</td>
<td>PcomA</td>
<td>3</td>
<td>Yes</td>
<td>1</td>
<td>Mild disorientation</td>
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<td>Right arm paresis, aphasia</td>
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<td>3</td>
<td>MCA L (mult), A.ophth. L, PCA L</td>
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<td>9</td>
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<td>MCA L</td>
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<td>1</td>
<td>Right-side hemiparesis</td>
<td>5</td>
<td>L temp</td>
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<tr>
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<td>R front</td>
<td>Yes, ACA L, PCA L/yes</td>
</tr>
<tr>
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<td>PcomA (mult.), ICA R, ICA L</td>
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<td>Yes</td>
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<td>5</td>
<td>ICA L</td>
<td>4</td>
<td>Yes</td>
<td>1</td>
<td>Left-side hemiparesis, aphasia</td>
<td>5</td>
<td>L front</td>
<td>NA/no</td>
</tr>
<tr>
<td>13</td>
<td>59</td>
<td>F</td>
<td>4</td>
<td>MCA R</td>
<td>4</td>
<td>Yes</td>
<td>1</td>
<td>Right-side hemiparesis, disorientation</td>
<td>2</td>
<td>R temp + L front</td>
<td>NA/yes</td>
</tr>
</tbody>
</table>

PcomA indicates posterior communicating artery; temp, temporal; ICA, internal carotid artery; AcomA, anterior communicating artery; front, frontal; mult, multiple; and ophth, ophthalmic.

the patient or nearest relative. All studies were performed in accordance with the Declaration of Helsinki as revised in Edinburgh in October 2000.

Patients
During the study period (June 2002 to February 2003), 13 patients (9 women, 4 men; mean age, 45.6 ± 14.1 years) with aneurysmal SAH were enrolled in a prospective study on cerebral metabolism monitored by bedside MD and PET. Patients >18 years of age with aneurysmal SAH who underwent surgical clipping of the aneurysm were eligible for the study. To minimize invasive interventions such as PET in asymptomatic control patients, we limited this group to 3 patients. Exclusion criteria were deranged coagulation parameters and low platelet count. Patients were managed according to standard protocols previously described. On the day of PET, physiological data (heart rate, FiO₂, blood gases, mean arterial blood pressure) were recorded. The partial pressure of carbon dioxide was 38.3 ± 3.0 mm Hg.

Diagnosis of Ischemia
The diagnosis of ischemia was clinically based on the following criteria (adapted from Lanzino et al11): (1) classic signs of symptomatic vasospasm (onset occurring from days 2 to 14 after SAH; worsening of headache; focal deficits not present on admission), (2) exclusion of rebleeding or hydrocephalus by CT, and (3) no other identifiable cause of neurological deterioration (eg, electrolyte disturbances). Symptoms of ischemia were confirmed by daily transcranial Doppler sonography (flow velocities >120 cm/s were regarded pathological)12 or angiography (Table 1). Control angiography was performed on day 8 or, in cases of neurological deterioration, earlier. Angiographically confirmed vasospasm was defined as any moderate to severe (>30%) narrowing of the cerebral vessel lumen.13

MD Technique
An MD catheter (membrane length, 10 mm; diameter, 0.6 mm; molecular weight limit, 20 000; CMA) was inserted immediately after clipping of the aneurysm into brain parenchyma of the respective vascular territory most likely to be affected by vasospasm, eg, the temporal lobe in patients with a middle cerebral arterial (MCA) aneurysm. MD was terminated when the patient was in a clinically stable condition (no further clinical deterioration expected).

Care was taken to avoid insertion of the catheter into macroscopically lesioned brain tissue adjacent to intracerebral hemorrhage. The catheters were inserted to a depth of 20 to 25 mm into predominantly white matter, tunneled through a burr hole of the bone flaps underneath the scalp, and fixed to the skin. Immediately after surgery, catheters were perfused with sterile Ringer’s solution at a flow rate of 0.3 μL/min with a microinfusion pump. The microdia-
Lysates were collected in microvials, exchanged hourly, and immediately analyzed at bedside in a mobile photometric, enzyme-kinetic analyzer for concentrations of glucose, pyruvate, lactate, and glutamate and frozen for later analysis of glycerol. The estimated recovery for the system is 0.65 to 0.72.14 MD data are presented as microdialysate concentrations.

**PET Technique**

PET scans were performed on an ECAT-Exact/921 PET scanner (47 slices; reconstructed in-plane resolution, 5 mm). The whole brain was imaged. For attenuation correction, a 10-minute transmission scan was acquired with a 68Ge/68Ga ring source. For each scan, 15 O-H2O (1 GBq in 6 to 8 mL normal saline) was administered as an intravenous bolus, followed by 20 mL saline at a flow rate of 2 mL/s. Forty-six frames were collected over an 11-minute period (35 at 2 seconds, 8 at 30 seconds, 3 at 120 seconds) beginning with the start of injection. Scan data were reconstructed by filtered back projection with corrections for attenuation, scatter, randoms, dead time, and decay and smoothed by a gaussian function with 7.3-mm full-width half-maximum.

**Image Analysis**

The CBF parametric images were produced by the method of Watabe et al.15 All images were smoothed with a gaussian function to a final image resolution of 6-mm full-width half-maximum and were fused by rigid transformation with a CT scan using an AMIRA software package (Konrad Zuse Zentrum) with implemented rigid transformation software, which applies an algorithm16 based on normalized mutual information.17

Location of the MD probe, which is not visible on PET images, was determined on CT scans. On the CT scan, a cylindrical volume of interest (VOI) was defined around the tip of the probe (MD-VOI) and the respective contralateral VOI on 3 contiguous CT slices (each 5 mm) and 15 mm in diameter, resulting in a volume size of 2.65 cm3. The extent of the VOIs was adjusted to the sampling volume of the MD catheter. For all patients, rCBF of the MD-VOI and all vascular territories was determined.

**Data Analysis**

Data are expressed as mean±SD unless otherwise stated. Relationships between MD values and CBF were explored through nonparametric methods (Spearman’s rank correlation test) to take account of nonlinear relationships between variables. Group comparisons were tested with the Mann-Whitney U test. Sensitivity and specificity were calculated with the χ2 test (Monte Carlo significance). A value of P<0.05 was considered significant (SPSS Inc).

**Results**

**Patient Characteristics**

Thirteen SAH patients were studied with PET (5 of them twice). Three patients were classified as asymptomatic (mean age, 44.7±14.6 years) and 10 patients as symptomatic (mean age, 49.5±11.7 years); both groups were comparable in age (P=0.69) and sex (P=0.29). Demographic data are summarized in Table 1. The first PET was performed within 8.9±6.5 days after initial bleeding. In 5 patients, a second PET was performed 13.8±4.8 days after SAH.

**PET Results**

Mean rCBF was significantly lower in the vascular territory of the operated side of the clipped aneurysm (P=0.048), MCA territory (P=0.034), and MD-VOI compared with the
TABLE 2. MD Parameters and Related rCBF in SAH Patients

<table>
<thead>
<tr>
<th></th>
<th>Asymptomatic</th>
<th>Symptoms of ischemia</th>
<th>Reference Levels</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/L</td>
<td>2.2±0.3</td>
<td>2.4±1.4</td>
<td>1.6±0.8</td>
<td>NS</td>
</tr>
<tr>
<td>Lactate, mmol/L</td>
<td>2.9±1.2</td>
<td>4.8±1.7</td>
<td>2.6±0.6</td>
<td>0.056</td>
</tr>
<tr>
<td>L/P ratio</td>
<td>18.5±3.5</td>
<td>40.7±37.0</td>
<td>24.1±4.2</td>
<td>0.018</td>
</tr>
<tr>
<td>Glutamate, μmol/L</td>
<td>4.8±3.6</td>
<td>30.6±39.4</td>
<td>2.0±0.6</td>
<td>0.056</td>
</tr>
<tr>
<td>Glycerol, μmol/L</td>
<td>65.5±42.5</td>
<td>90.0±43.1</td>
<td>82±44</td>
<td>NS</td>
</tr>
<tr>
<td>rCBF&lt;sub&gt;MD&lt;/sub&gt;, mL·100 g&lt;sup&gt;−1&lt;/sup&gt;min&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>54.7±21.8</td>
<td>33.6±11.4</td>
<td>&gt;20</td>
<td>0.064</td>
</tr>
</tbody>
</table>

rCBF in Relation to Symptoms
Symptomatic patients had significantly lower rCBF values on the operated side compared with asymptomatic patients (Figure 1). Additionally, mean rCBF of the operated side was lower in symptomatic patients compared with the contralateral side (MD-VOI, P<0.02; clipped territory, P<0.084; anterior cerebral artery [ACA] territory, P<0.048; MCA territory, P<0.048; posterior cerebral artery [PCA] territory, P<0.068), whereas asymptomatic patients had no vascular territorial asymmetries. In 5 patients with sequential PET studies, rCBF values were slightly higher in the second PET (P=NS; Figure 2). Symptoms of these patients changed considerably from PET 1 to 2 (improvement, n=2; deterioration, n=2; almost unchanged, n=1).

MD Data in Relation to Symptoms
Because cerebral metabolism is highly variable, depending on the neurological condition of the patient,7,18 MD data during asymptomatic and symptomatic intervals (determined as mean of 3-day medians of hourly measured parameters) were compared. This analysis was used because asymptomatic patients develop vasospasm and typically secondarily deteriorate with a complete change in metabolism.9 In symptomatic intervals, the L/P ratio (P<0.018) and in trend the extracellular lactate (P<0.056) and glutamate (P<0.056) were significantly higher compared with asymptomatic intervals (Table 2). The L/P ratio was most sensitive and specific in indicating symptoms of ischemia (sensitivity, 0.82; specificity, 1.0), whereas glutamate was less specific (sensitivity, 0.82; specificity, 0.5). Lactate was more specific than glycerol (lactate: sensitivity, 0.64; specificity, 0.75; glycerol: sensitivity, 0.73; specificity, 0.5); glucose had the lowest sensitivity (0.27; specificity, 1.0).

PET-MD
The rCBF within the MD-VOI and the simultaneously measured MD data were compared (Figure 3). There was a significant relation between rCBF and glutamate (r=-0.66, P=0.014) and glycerol (r=-0.62, P=0.038) and a weak correlation of rCBF with lactate (r=-0.49, P=0.063), whereas glucose (r=-0.40, P=0.111) and the L/P ratio (r=-0.10, P=0.385) were not related to rCBF.

In a second step, we wanted to identify the MD parameter most specific and sensitive for indicating cerebral ischemia. Thresholds were defined as follows: pathological MD levels: glutamate >5 μmol/L; lactate >4 mmol/L; L/P ratio >25; glycerol >70 mmol/L; critically reduced rCBF=rCBF<20 mL·100 g<sup>−1</sup>min<sup>−1</sup>. The sensitivity to detect cerebral ischemia was highest for glutamate and lactate (both 1.0), followed by the L/P ratio and glycerol (both 0.67), and was very low for glucose (0.33) and pyruvate (0.0). Specificity was generally low and highest for lactate (0.75), the L/P ratio, glycerol, and glucose (0.5) and lowest for glutamate (0.38) and pyruvate (0.25).
Discussion

First, a reduction in rCBF determined by PET was indicated best by the MD parameter glutamate, followed by glycerol. Second, the L/P ratio was the most specific and sensitive parameter to reflect clinical ischemia, but only during longer periods of ischemia. In ischemic patients, rCBF was significantly lower in the clipped territory and MD region compared with asymptomatic patients, indicating a clinical relevance for the observed metabolic changes. Nevertheless, in most symptomatic patients, the measured rCBF values were above the critical threshold of ischemia. We conclude that clinically relevant regional metabolic derangements occur already above an rCBF of 20 mL·100 g⁻¹·min⁻¹.

This is the first study demonstrating a clear correlation between rCBF determined by PET and simultaneously measured extracellular glutamate and glycerol in SAH patients. Sensitivity for glutamate in detecting a critically reduced rCBF (<20 mL·100 g⁻¹·min⁻¹) was high. However, glutamate was rather unspecific for detecting a critical rCBF, which indicates that glutamate changes possibly occur already above the threshold of ischemia. Increases in glutamate have been found to correlate with severe ischemia in severe-head-injury patients but may also be caused by other mechanisms such as transmitter release and unspecific leakage from injured cells. Nevertheless, clinical studies with SAH patients support the hypothesis that glutamate plays a major role as an early marker of impending ischemia.

Glycerol, an end product of phospholipid breakdown and marker of cell membrane degradation, was the second parameter that correlated with rCBF, a finding that was surprising because, in most reports, increases in glycerol were described during development of cerebral infarction. Our finding is supported by results of Enblad and coworkers, who observed a marked sustained elevation of glycerol directly after MCA occlusion that normalized only in penumbra regions but not within the infarct core.

Although the main objective of this study was to compare MD with PET measurements during the PET scans, this period of MD monitoring represented only a small proportion of the total MD monitoring in these patients. This is reflected in the discrepancy of our findings that the L/P ratio was the most sensitive and specific MD parameter to indicate symptoms of ischemia, but not related to CBF. The lack of correlation of the L/P ratio during simultaneous PET-MD monitoring is possibly the expression of a compensated energy metabolism that may deteriorate, depending on the clinical course and duration of a reduced rCBF. This observation is substantiated by results of previous studies in which patients with delayed ischemic neurological deficits first developed pathologically high glutamate and lactate levels and secondarily an increase in the L/P ratio. The role of lactate in ischemia is still unclear. Lactate may play a role as a substrate for neurons in preference to glucose, driven by glutamate-induced glycolysis. A PET study in patients with severe head injury (n = 17) did not find any correlations between MD parameters and rCBF. The authors explain this finding with the fact that most patients had CBF values higher than the threshold for ischemia (18 mL·100 g⁻¹·min⁻¹). Similarly, we measured in only 3 symptomatic patients rCBF values <20 mL·100 g⁻¹·min⁻¹; high glutamate, lactate, and glycerol values; but a normal L/P ratio. In these patients, hypodense regions within the monitored vascular territory of the aneurysm, suggesting focal infarction, were visible on late CT scans. When these patients had longer intervals of ischemia, the L/P ratio was significantly elevated, indicating that the duration and severity of ischemia are important. Interestingly, mean rCBF was significantly lower in patients with symptoms of ischemia even when rCBF values were not below the critical threshold of ischemia (<20 mL·100 g⁻¹·min⁻¹) in most patients. In our view, this regional asymmetry possibly indicates a high risk for a further clinical deterioration, eg, after a decrease in mean arterial blood pressure.

Our results support the value of the MD technique for online monitoring of cerebral metabolism, but there are several pitfalls. The method is a feasible and safe real-time monitoring instrument in SAH patients. Because the technique using CMA 600 can measure only 4 substances (choosing from glucose, lactate, pyruvate, glutamate, glycerol, urea) at a time, the most relevant for detection of ischemia have to be selected. So far, we recommend in SAH patients to monitor energy metabolism (glucose, lactate, pyruvate) and glutamate as suggested markers of CBF changes. Furthermore, the location of the MD probe is crucial, and care should be taken to avoid insertion into a clot. The responses to ischemia in gray and white matter are probably different, and histopathological data suggested a greater susceptibility for infarction of the gray than the white matter for a given degree of rCBF reduction.

Conclusions

During PET measurements, reduced rCBF correlates best with glutamate, followed by glycerol, whereas the L/P ratio is sensitive only after longer periods of ischemia. Our data demonstrate that clinically relevant regional metabolic derangements occur already above an rCBF of 20 mL·100 g⁻¹·min⁻¹. Future research should focus on identifying alternative causes of metabolic derangement in SAH patients and optimal treatment management in these patients.

Acknowledgments

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References

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